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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

We hypothesized that genes that are differentially expressed as a result of the decreased IGF-I receptor gene expression seen in metastatic prostate cancer contribute to prostate cancer progression. IGF-I receptor target genes may include metastasis-promoting or suppressing genes that could constitute valuable diagnostic markers or therapeutic targets. To evaluate this hypothesis, we proposed three specific aims: 1) Identification of genes that are differentially regulated in otherwise isogenic metastatic vs. non-metastatic prostate epithelial cells; 2) Identification of proteins that are differentially secreted in the cell lines used in aim 1, and 3) Assessment of the differential expression of these genes and gene products in laser-microdissected samples. We have used microarray gene profiling to characterize differentially expressed genes and have used SELDI-TOF mass spectrometry to identify proteins that are differentially secreted into conditioned media. We have additionally initiated a 3-dimensional culture system to grow prostate cells in a microgravity environment that more accurately replicates in vivo cell organization and phenotype. We are now preparing to quickly repeat our microarray and SELDI-TOF analyses in 3-D culture before proceeding to the validation of differential expression in clinical samples using prostate tissue arrays.

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Introduction:

The insulin-like growth factor (IGF) signaling system plays an important role in the initation and progression of prostate cancer. High circulating levels of IGF-I are associated with an increased risk of developing prostate cancer, and constitutive expression of IGF-I in the prostate epithelium of transgenic mice results in neoplasia. The actions of IGF-I are mediated through activation of the IGF-I receptor, a transmembrane tyrosine kinase that is highly expressed in normal prostate epithelium and immortalized prostate epithelial cell lines. A significant *decrease* in IGF-I receptor expression is seen in metastatic prostate cancer cell lines, human metastases, and metastatic lesions from transgenic mouse models of prostate cancer. Retroviral re-expression of the IGF-I receptor in metastatic prostate cancer cells reduces their tumorigenicity and metastatic potential. These data suggest that, while IGF-I action may contribute to the initiation of prostate cancer, a subsequent loss of IGF responsiveness secondary to reduced IGF-I receptor expression is necessary for progression to advanced disease. This requirement for decreased IGF-I responsiveness may reflect the ability of the activated IGF-I receptor to exert differentiative as well as proliferative effects. Our hypothesis is that genes that are differentially expressed as a result of the decreased IGF-I receptor gene expression seen in metastatic prostate cancer contribute to prostate cancer progression. Specifically, IGF-I receptor target genes may include metastasis-promoting or suppressing genes that could constitute valuable diagnostic markers or therapeutic targets. To evaluate this hypothesis, we proposed three specific aims: 1) Identification of a select subset of genes that are differentially regulated in otherwise isogenic metastatic vs. non-metastatic prostate epithelial cells that differ solely in the level of expression of the IGF-I receptor; 2) Identification of proteins that are differentially secreted in the cell lines used in aim 1, and 3) Assessment of the differential expression of these genes and gene products in laser-microdissected samples from normal prostate, adenocarcinoma, and metastatic lesions. The studies of specific aim 1 employ microarray gene profiling of metastatic prostate epithelial cells and their non-metastatic counterparts that are re-expressing the IGF-I receptor from a retroviral construct. The studies of aim 2 employ surface-enhanced laser desorption-ionization/time-of-flight (SELDI-TOF) mass spectroscopy to identify proteins that are differentially present in the conditioned media of the two cell types utilized in aim 1. Subsequent tandem mass spectroscopy analyses will be performed to generate proteolylic cleavage patterns or peptide sequences for gene identification. The studies of specific aim 3 will evaluate the expression of IGF-I receptor target genes in a series of human clinical samples using quantitative real-time RT-PCR analyses. The proposed studies address a critical aspect of prostate cancer, i.e., factors that contribute to the development of advanced disease. The studies underway employ an innovative, integrated, gene and protein profiling approach in a novel, carefully defined and highly controlled model system to identify genes and gene products that regulate metastasis.

Body:

The approved statement of work included three tasks that were to be initiated in the first 12 months of funding. These tasks were in support of aims 1 and 2 of the proposed project. These will be discussed in turn.

Task 1:Completion of microarray analysis of genes differentially expressed in LISN and LNLG cells that express different levels of IGF-I receptor and which are, respectively, non-metastatic and metastatic in nude mouse xenografts. We have completed this analysis using three independent RNA preparations from each cell line grown in defined medium with 1% FBS and have analyzed each sample using triplicate arrays that each contain >12,000 sequence-verified, non-redundant human cDNA clones. Data have been analyzed by accepted means of normalization, statistical verification and false-discovery rate analyses. These data demonstrate that there are specific genes that are constantly differentially expressed in LISN and LNLG cells. We have recently acquired a rotary cell culture system for three-dimensional (3-D) cultures of cells in a microgravity environment. This NASA-designed apparatus had been used to propagate cells under conditions that alter them to form 3-D structures that may be more similar to the in vivo situation (1,2). Indeed, a recent report has shown that tumor cell lines grown under microgravity conditions display a phenotype that is much more similar to that of clinical samples in situ than cells grown in monolayer cultures (3). This system has been used in several previous studies to grow prostate cells in particular (4-7). We would like to propose to use this novel culture

system to repeat the array analysis that constituted task 1, since we feel that the data obtained will be significantly more relevant than that obtained with standard monolayer culture.

Task 2:Completion of preliminary SELDI-TOF analysis of conditioned media from M12-LISN and M12-LNLG cultures. We have completed several profiles using normal phase protein array chips in the Ciphergen PBSII SELDI-TOF platform, and have identified a number of molecular weight species that appear to be differentially expressed and secreted by the two cell lines. For further investigation however, we would prefer to generate additional profiles of cells grown in 3-D cultures, for the reasons cited above for task 1. This will be a relatively simple change to implement, since we can easily obtain RNA and conditional media from the same rotary cultures.

Task 3:Generation of probes for Northern and/or RPA analysis and verification of differential gene expression in M12-LISN and M12-LNL6 cells. We have begun to design primers of generation of cDNA probes for some of the robustly differentially expressed genes identified in the array studies of task 1, but will first determine which of these are differentially expressed in these cell lines grown in 3-D culture before proceeding further.

Tasks 4-8, slated for performances in years 2 and 3, remain largely unaltered, except that through our collaborator, Dr. Stephen Plymate, we will now have access to prostate tissue arrays containing over 100 samples ranging from normal prostate epithelium through Gleason grade 9 tumors. This will facilitate the analysis of differential expression of target genes for which suitable antibodies are available, and will be preferable to analyzing individual specimens on separate slides, since all the samples on the tissue array are processed simultaneously.

Key research accomplishments:

- Demonstration of differential gene expression profiles in M12-LISN and M12-LNLG cells expressing different levels of IGF-I receptor.
- Demonstration of differential secreted protein expression in M12-LISN and M12-LNLG cells.
- Establishment of 3-D cell culture system for M12-LISN and M12-LNLG cell culture.

Reportable outcomes: None for this funding period.

Conclusions: We have shown that differences in IGF-I receptor gene expression that are sufficient to alter metstatic capacity are sufficient to alter gene expression and secreted protein expression profiles in monolayer cell culture. There is an increasing appreciation that findings made in monolayer cell culture may not accurately reflect the molecular situation in vivo, as compared to what can be achieved with 3-D culture approaches (3, 8-10). We are, however, well positioned to quickly reevaluate differential gene and secreted protein expression in 3-D culture, which will allow us to then proceed with the additional components of the statement of work using more promising candidate IGF-regulated genes.

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Appendices: None.